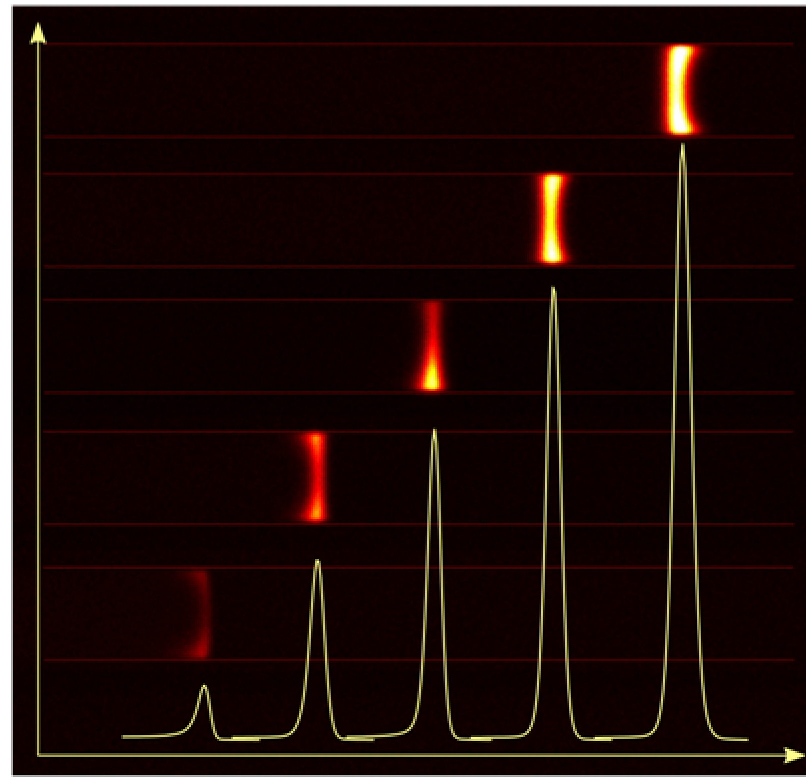
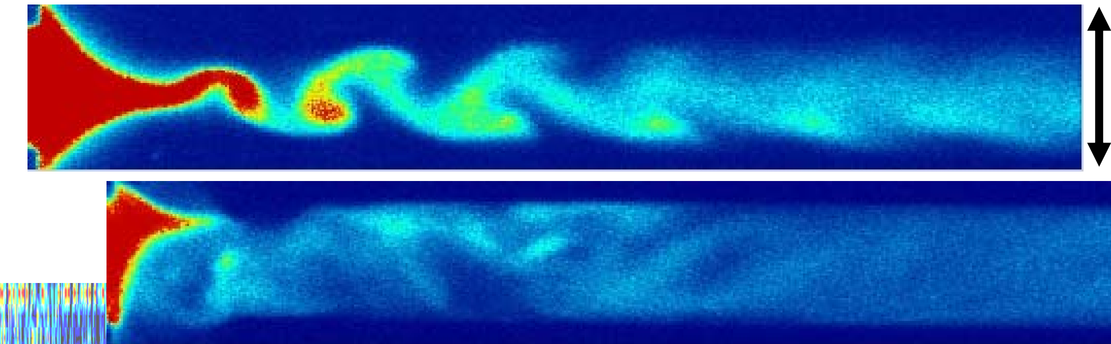
Making Shockwaves in Microfluidics:
The Physics of Isotachophoresis and Its Applications



Juan G. Santiago
Stanford Microfluidics Laboratory
Mechanical Engineering Dept.
Stanford University

Intro to Stanford Microfluidics Lab

Electrokinetic instabilities:

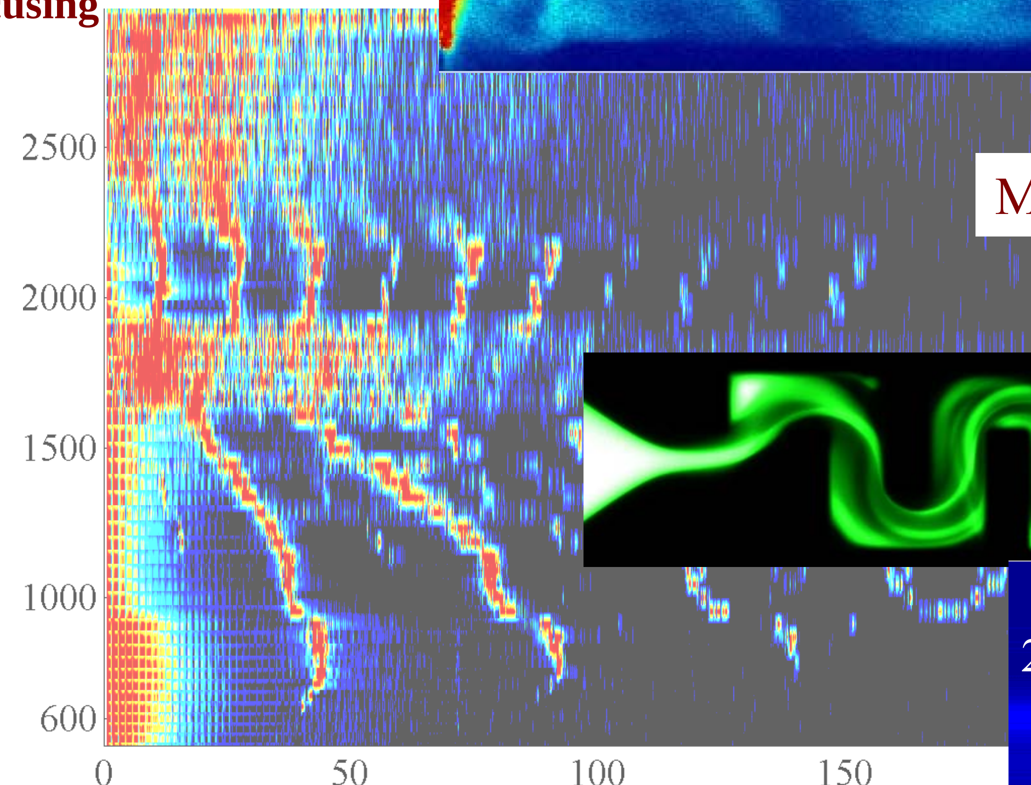


Activities:

Miniature Bioanalytical Systems
- **Capillary zone electrophoresis**
- **Capillary isoelectric focusing**
- **PCR**
- **DNA separation**

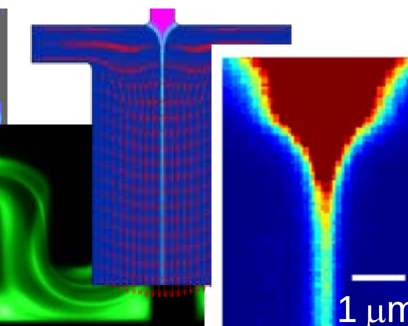
Microflow Devices
- **Micromixers**
- **Electroosmotic pumps**
- **Miniature fuel cells**
- **On-chip 2D assays**

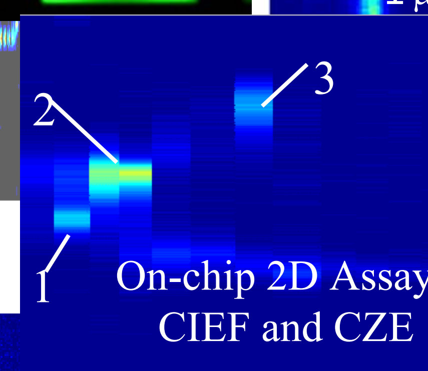
Applications
- **Drug discovery**
- **Bioweapon detection**
- **Proteomics**
- **BW detection**
- **Electronics cooling**
- **Drug delivery**
- **Power generation**

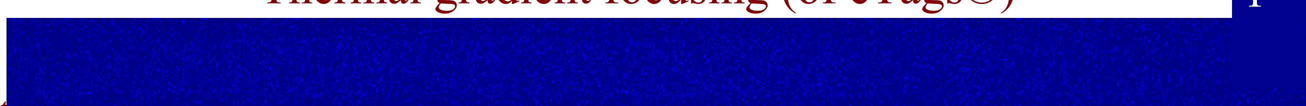


Thermal gradient focusing (of eTags©)

Micromixers:







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- ITP process and visualizations
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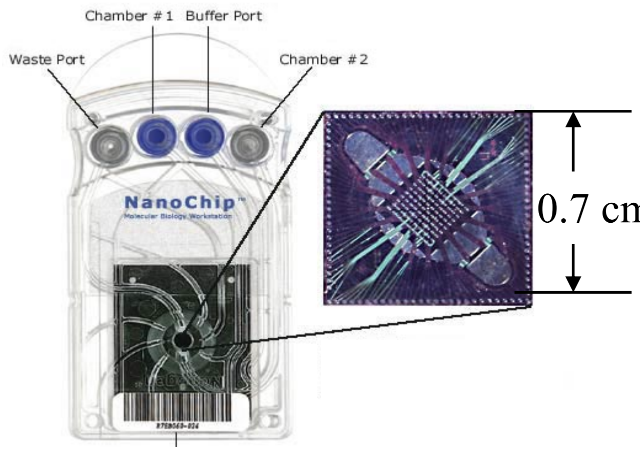
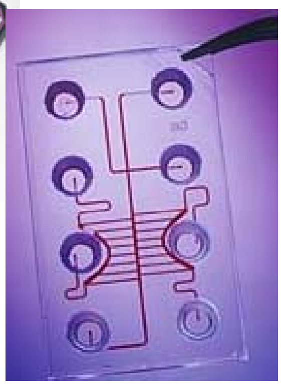
Microfluidics

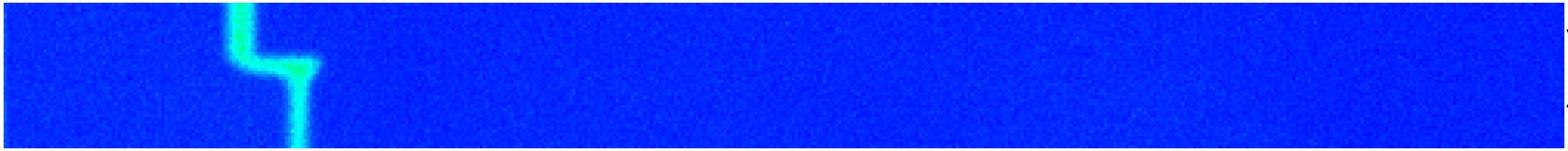
- Applications
  - ~~Bio-weapon detection~~ Fear
  - ~~Pharmaceuticals/drug discovery~~ Greed
  - ~~Environmental monitoring~~ Pride
  - Point-of-care medical diagnostics
- Challenges and Advantages
  - Reduced reagent use
  - Specificity, sensitivity
  - Integration and automation
  - Portability and robustness
  - Potential for parallel analyses



www.nanogen.com



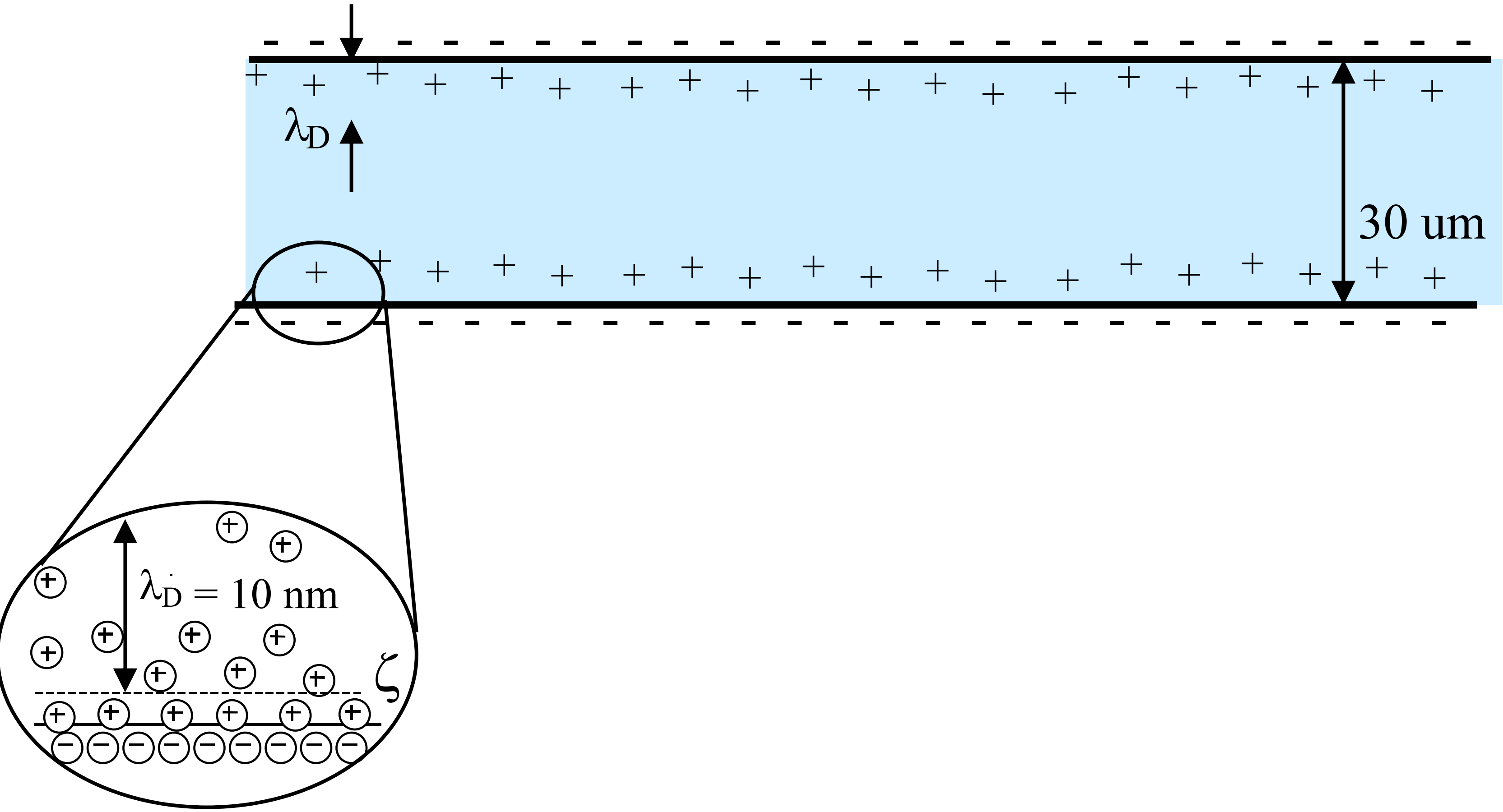
Caliper/Agilent Tech
www.caliperls.com



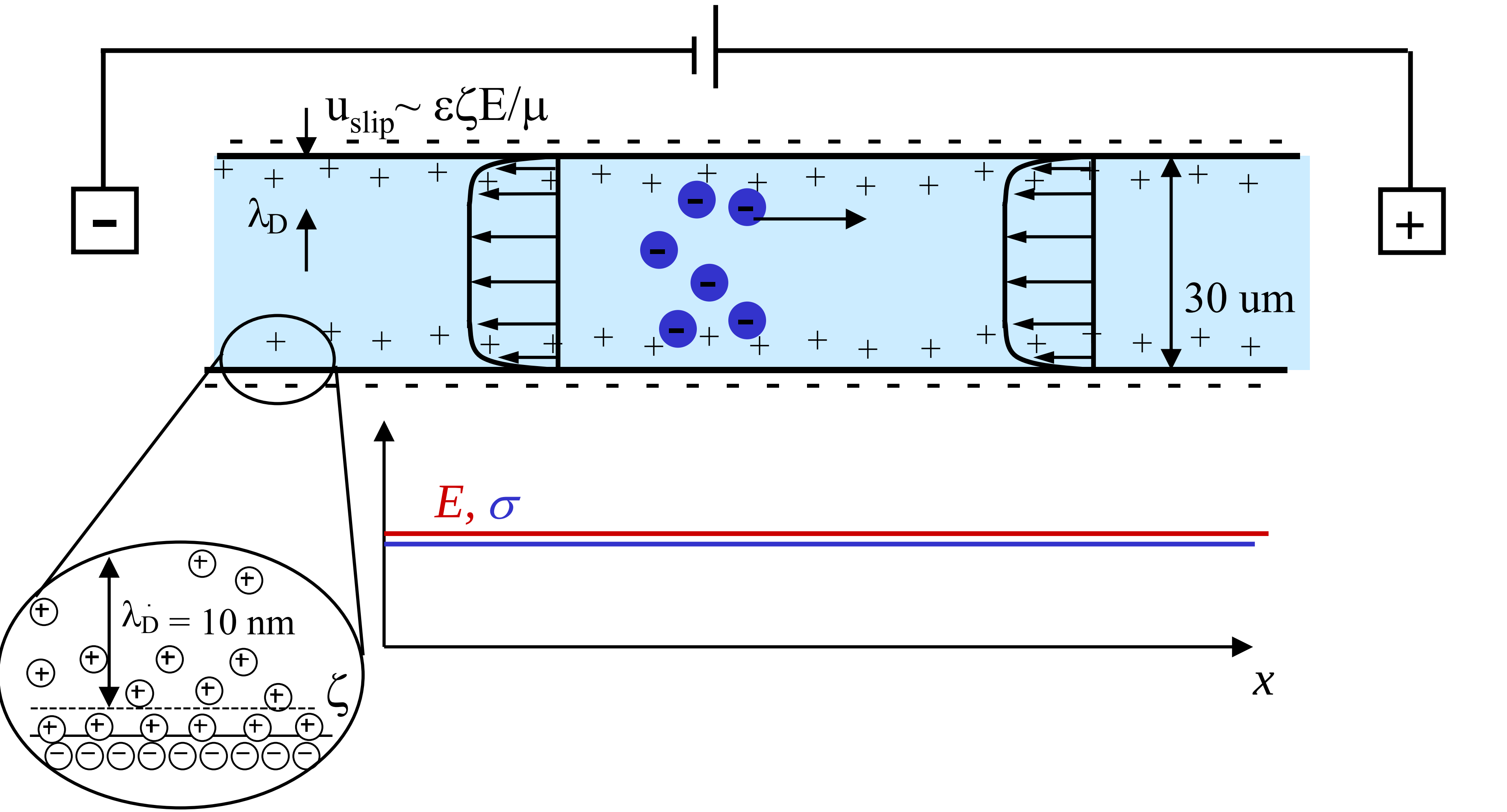
30 um

On-chip capillary electrophoresis

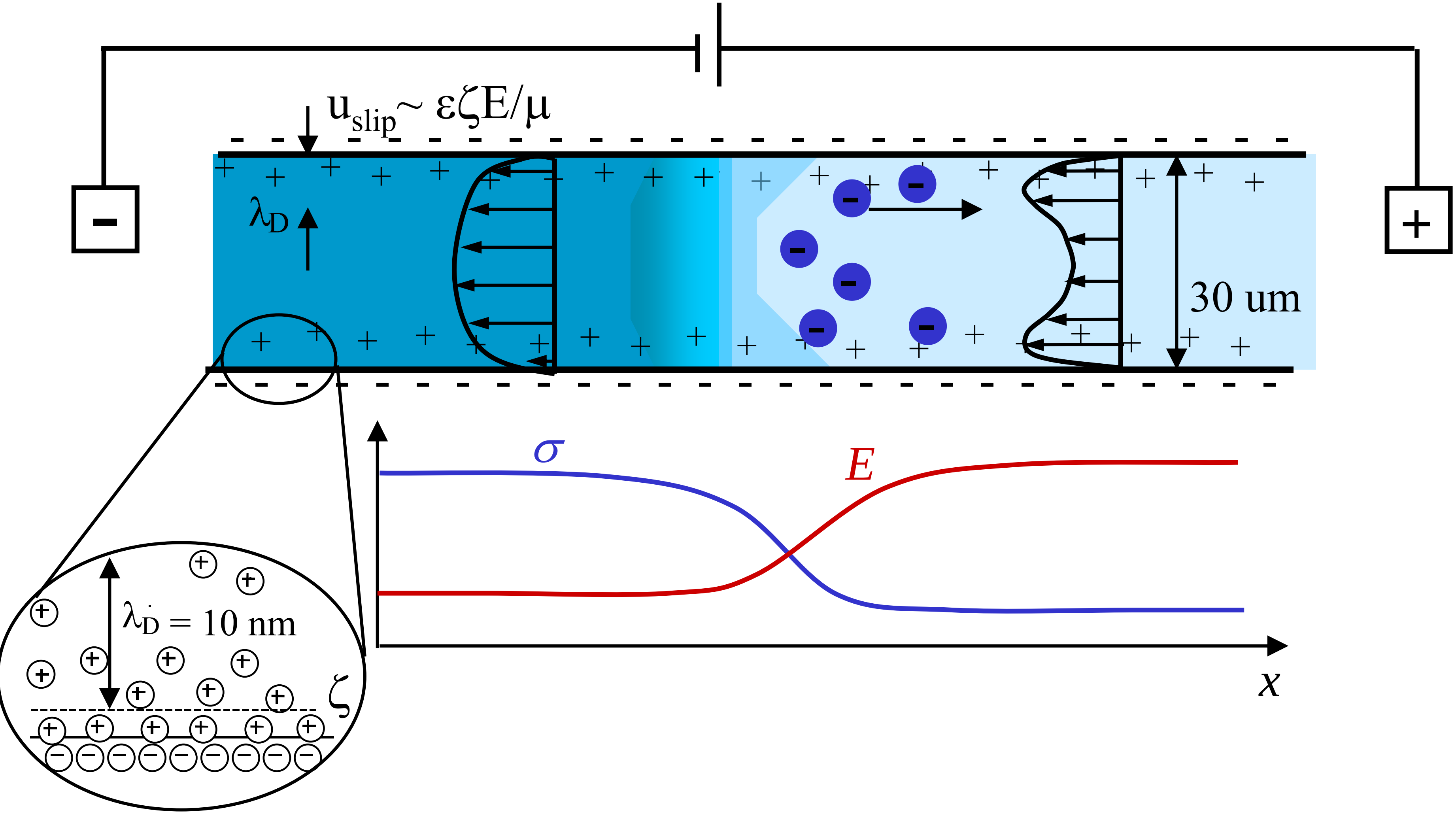
Electrokinetic Transport



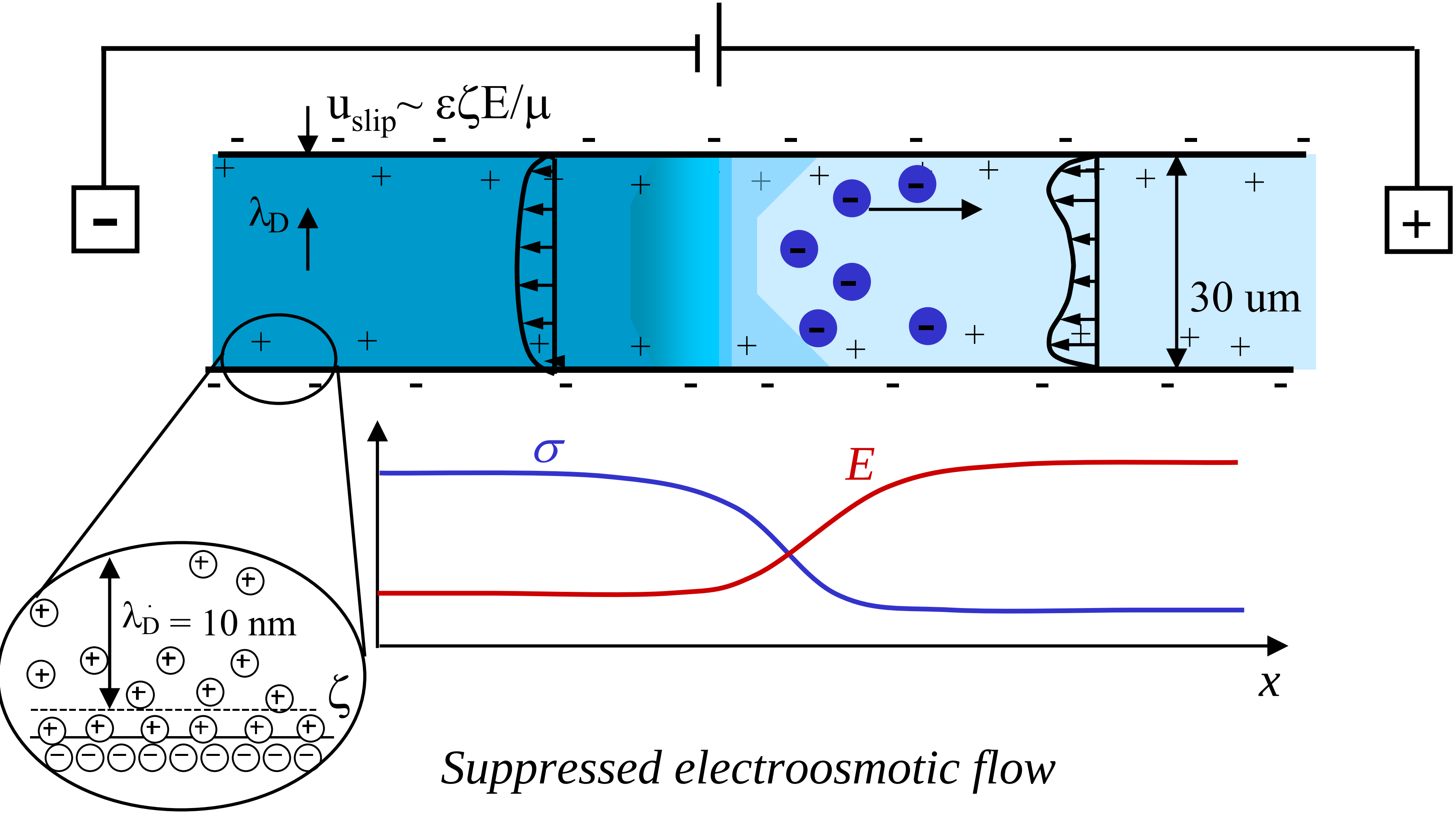
Electrokinetic Transport



Electrokinetic Transport



Electrokinetic Transport



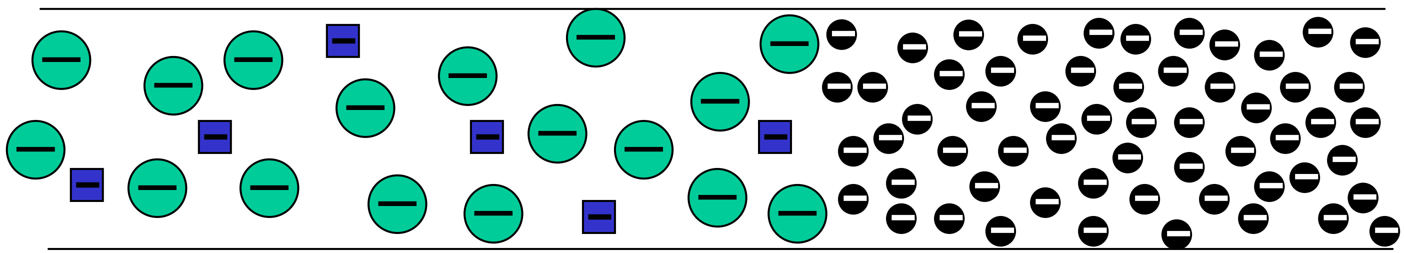
Suppressed electroosmotic flow

Isotachophoresis (ITP) Background

- 20,000+ citations; currently ~1.5 times/week
- **Kohlrausch**: Ion theory, KRF function (1897)
- **Longsworth**: Moving boundary electrophoresis for determining transference number of strong electrolytes (1930)
- **Tiselius** (1930); **Martin** (ITP, 1942), **Alberty** (weak electrolytes, 1949)
- **Gebauer P and Bocek P:** Relationship for order of ITP zones and their stability (1983)
- **Saville DA et al.:** Generalized model for electrophoresis, ITP, IEF including multispecies, buffer reactions and diffusion (1983)
- **Wainright et al.:** On-chip application to eTags, 530 fold, (2002)
- **Gas et al.**: SIMUL – free dynamic simulator of electrophoresis (2005)
- **Jung, Santiago**, et al. (2006) – Million fold ion focusing in 2 min, 100 attomolar sample detection

Isotachophoresis:

Single species focusing



50 um

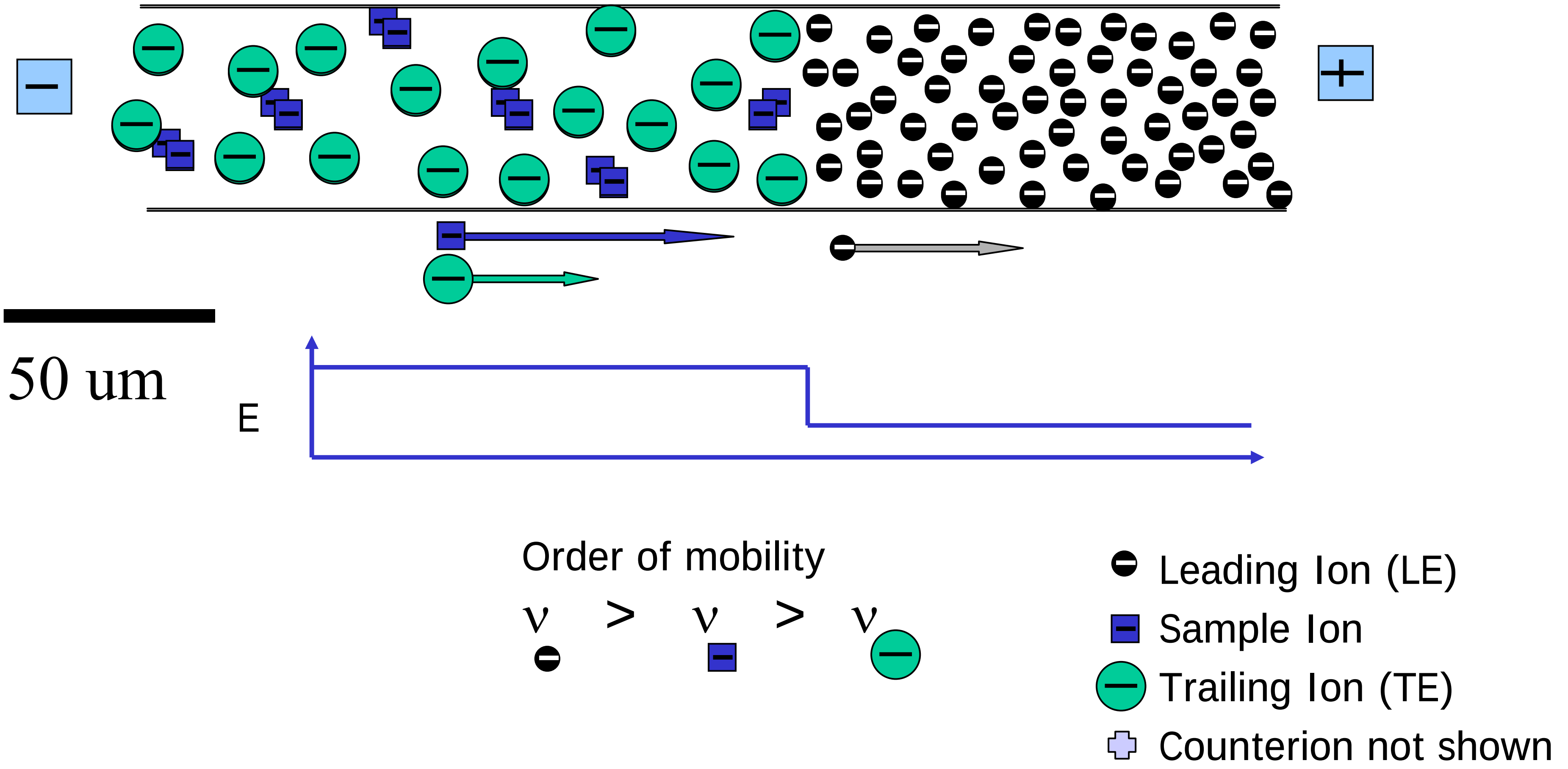
Order of mobility

ν (LE) > ν (Sample) > ν (TE)

- Leading Ion (LE)
- Sample Ion
- Trailing Ion (TE)
- Counterion not shown

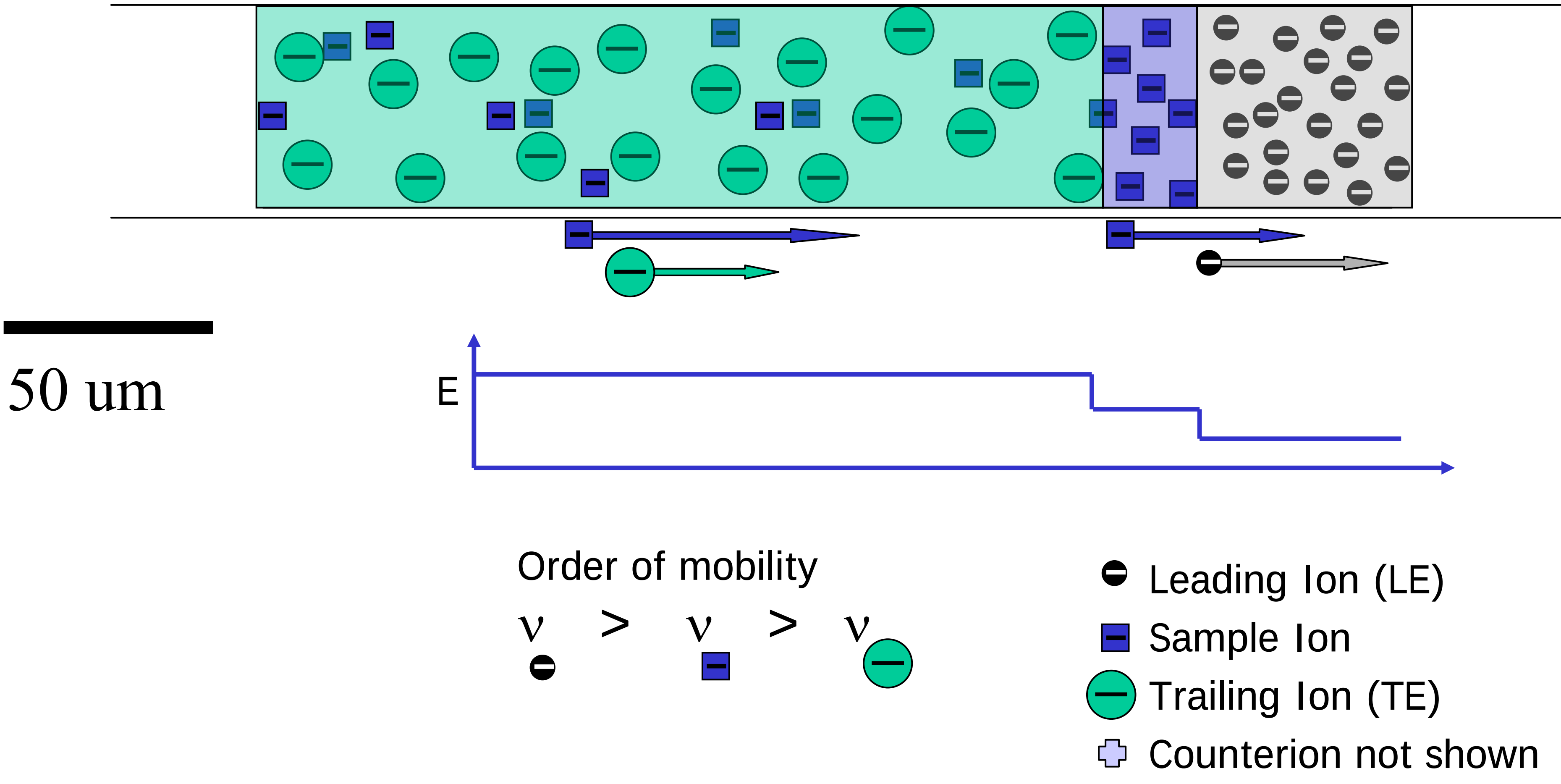
Isotachophoresis:

Single species focusing



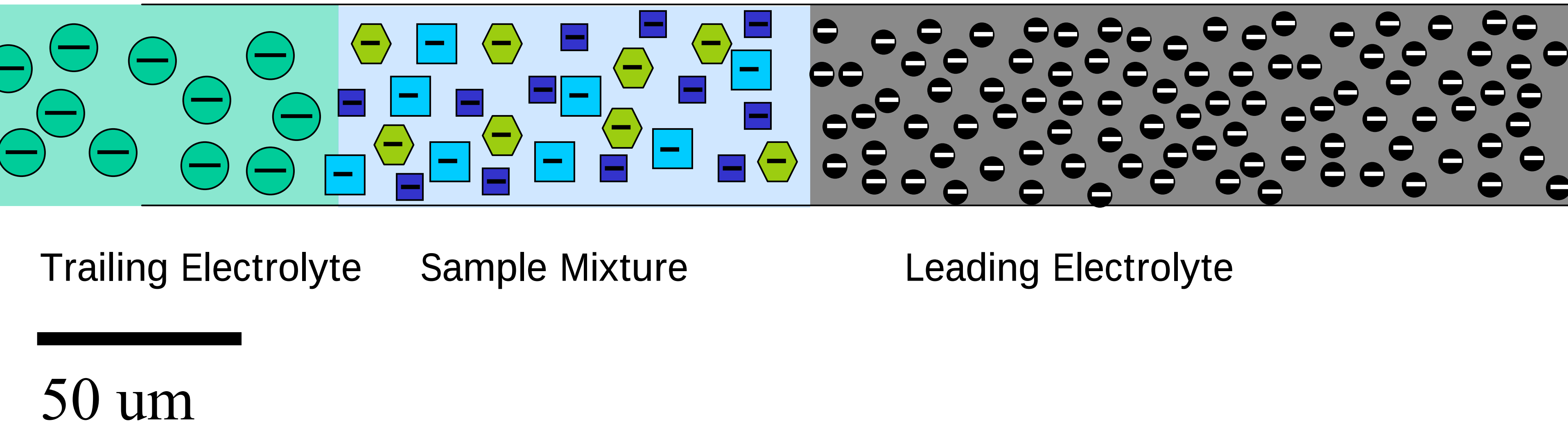
Isotachophoresis:

Single species focusing

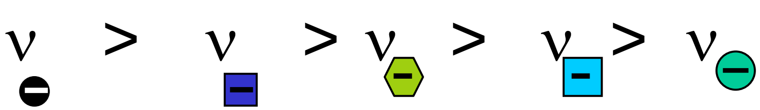


Isotachophoresis:

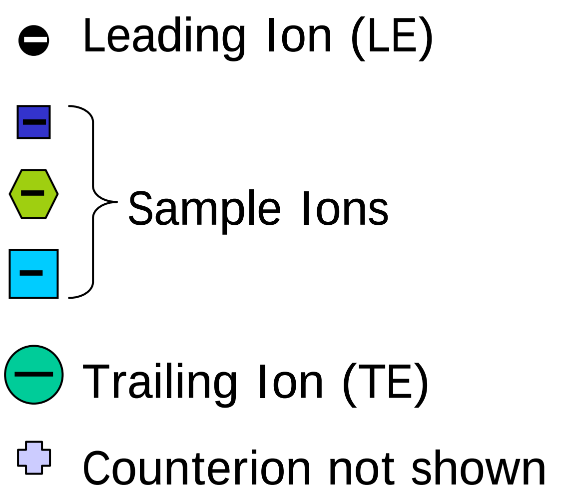
Multispecies focusing



Order of mobility (fully ionized)



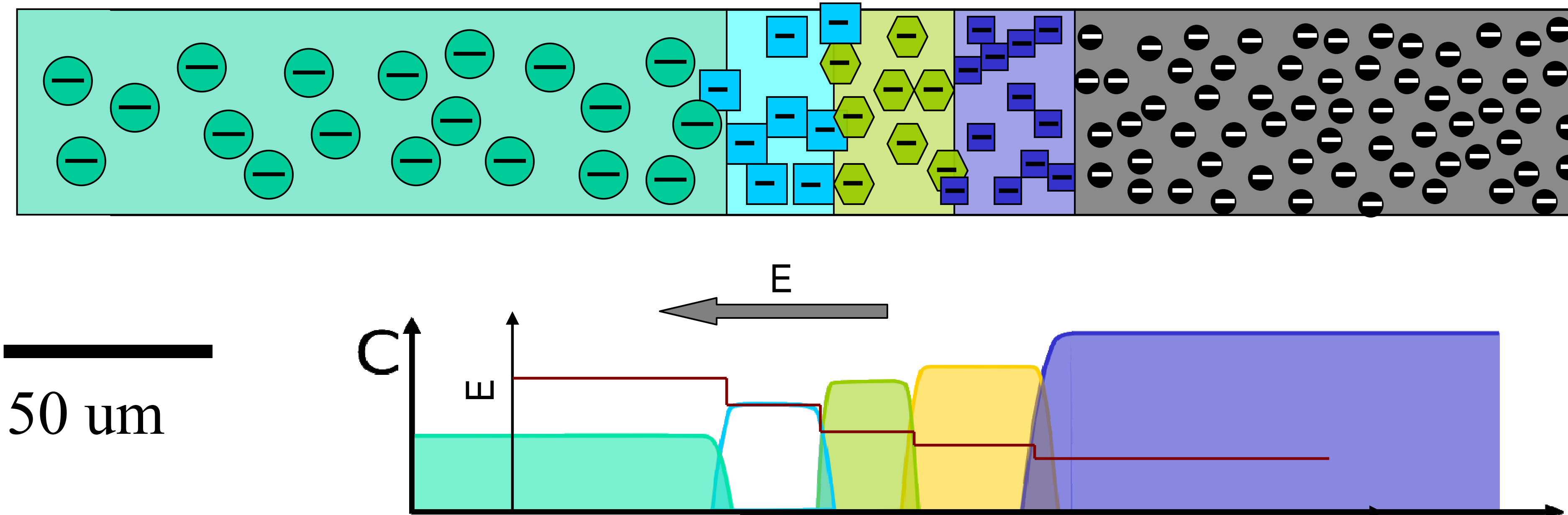
Electromigration velocity = ν E = Constant



"Iso"-"tacho"-"phoresis"

Isotachophoresis:

Multispecies focusing



Order of mobility (fully ionized)

ν > ν > ν > ν > ν

Electromigration velocity = ν E = Constant

Leading Ion (LE)

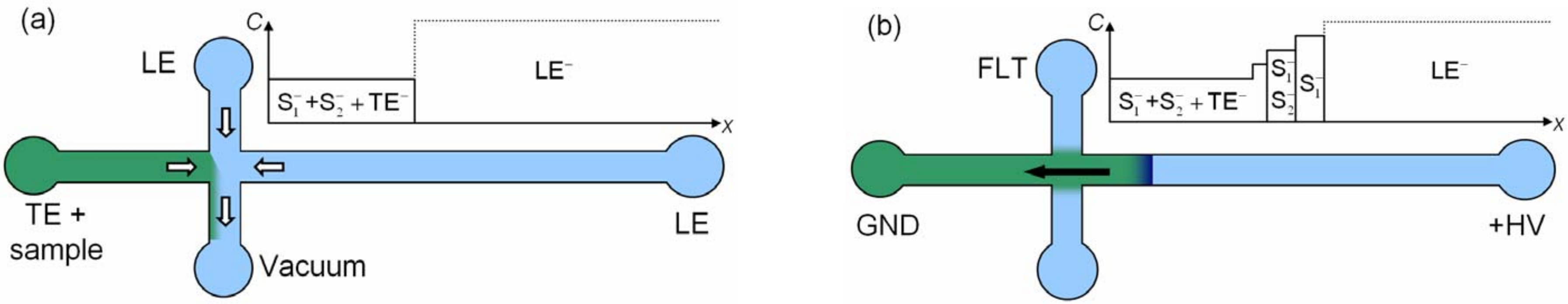
Sample Ions

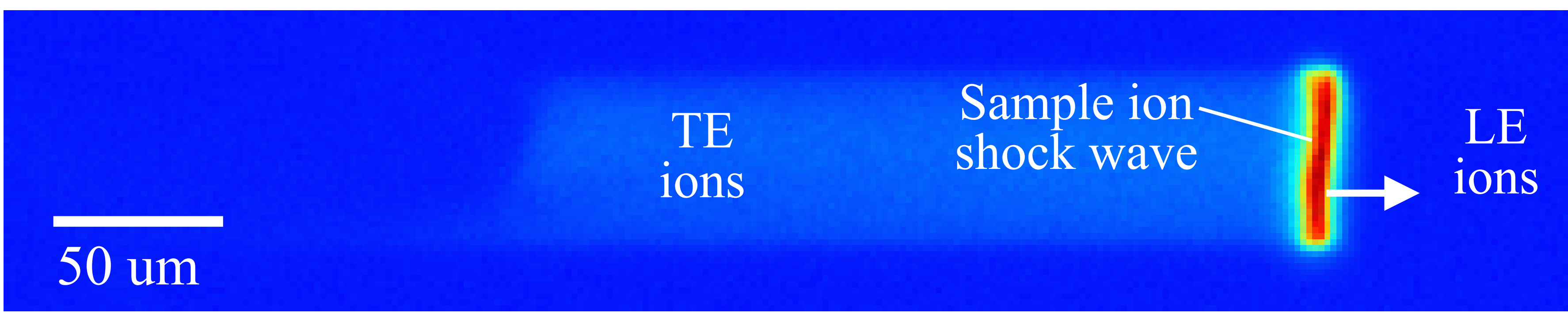
Trailing Ion (TE)

Counterion not shown

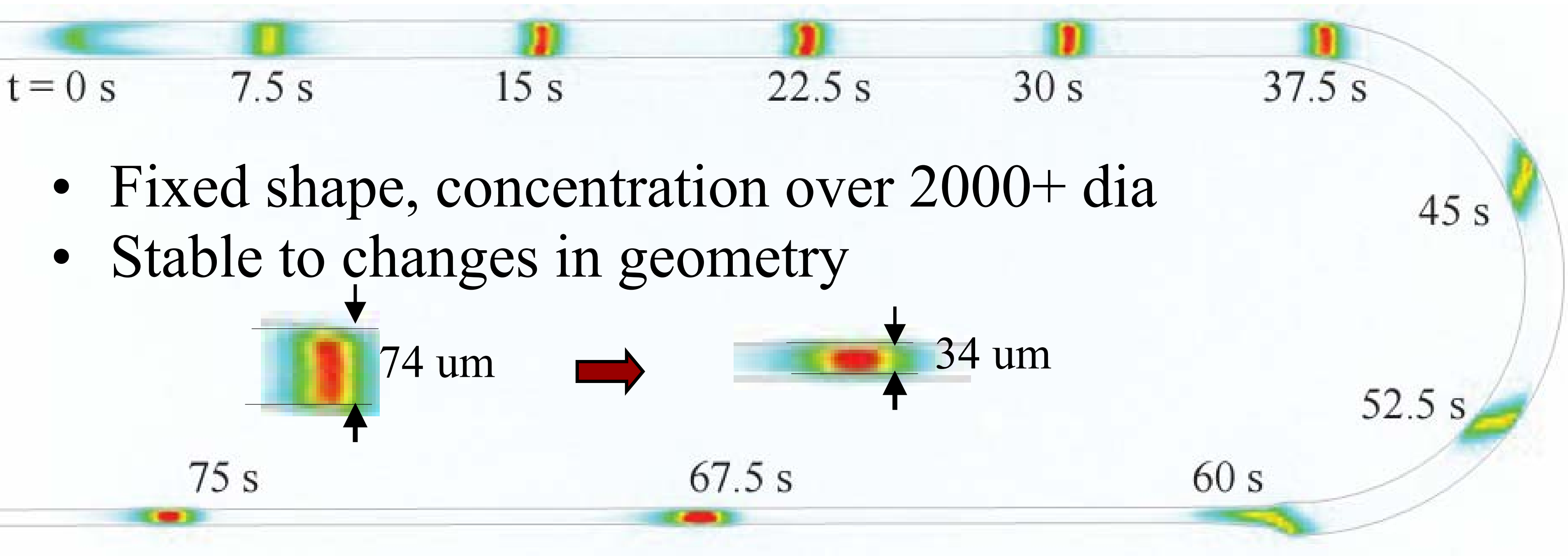
"Iso"-"tacho"-"phoresis"

ITP Experiments On-Chip





Stability of ITP Zones



- Fixed shape, concentration over 2000+ dia
- Stable to changes in geometry

- Typically EHD stable to ~1 kV/cm
- Stable to perturbations in electric field and pressure:

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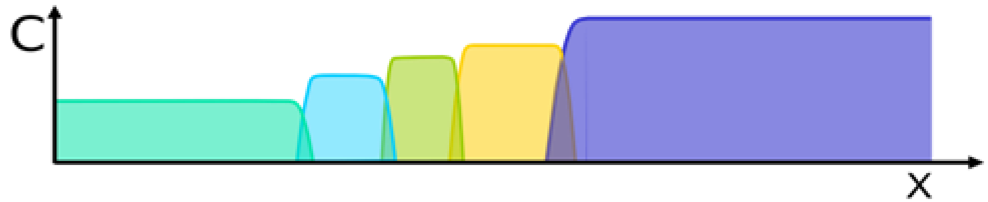
ITP Models



- Formulations

1D flow

$$\frac{\partial c_i}{\partial t} + \bar{u}\cdot\nabla c_i = \nu_i z_i F \nabla\cdot(c_i \nabla\phi) + \left(D_i \nabla^2 c_i\right) + R_i \Rightarrow \frac{\partial c_i}{\partial t} = \frac{\partial}{\partial x}\left[D_i \frac{\partial c_i}{\partial x} - u c_i - F z_i \nu_i \frac{\partial \phi}{\partial x} c_i\right] + R_i$$

Area averaging

$$-U_{ITP}\frac{\partial\langle C_i\rangle}{\partial x} = \frac{\partial}{\partial x}\left[-z_i F \mu_{eph,i}\langle C_i\rangle\langle E_x\rangle\right] + \left(D_i + \frac{(\mu_{eof}E_o)^2 a^2}{48 D_i}\right)\frac{\partial^2\langle C_i\rangle}{\partial x^2}$$

- Method of characteristics
- Perturbation model
- Shock capture model
  - N weak electrolytes
  - Real-time control of multiple input streams
  - Optimization
  - Area-averaging and Taylor dispersion

No advection

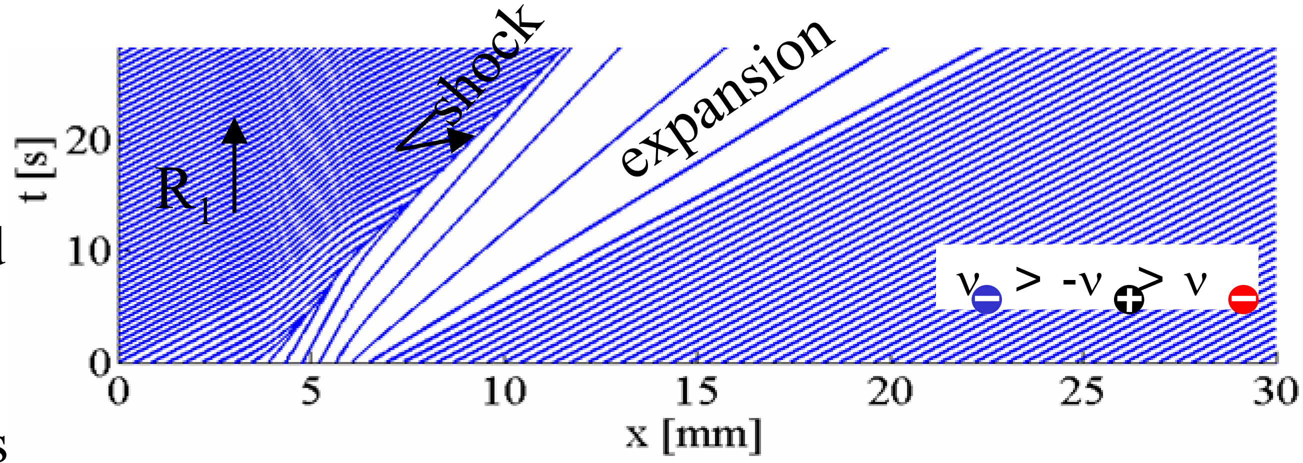
$$\frac{\partial c_i}{\partial t} = \frac{\partial}{\partial x}\left[D_i \frac{\partial c_i}{\partial x} - F z_i \nu_i \frac{\partial \phi}{\partial x} c_i\right] + R_i$$

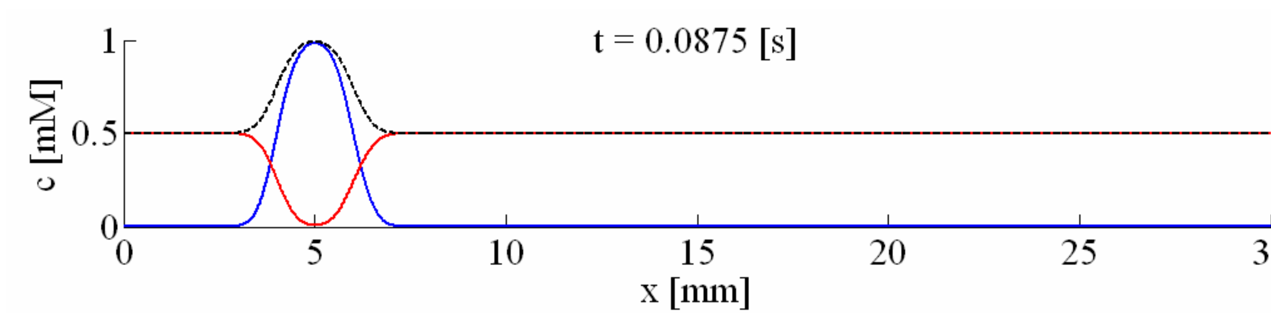
No diffusion, fully ionized

$$\frac{\partial c_i}{\partial t} = \frac{\partial}{\partial x}\left[F z_i \nu_i \frac{\partial \phi}{\partial x} c_i\right]$$

Preliminary work: Characteristics model

- Negligible diffusion, advection
- Assume fully ionized (relaxed charge, net neutrality)
- Shocks & expansions (electromigration dispersion)
- Initial conditions "remembered" via KRF
- 3 species:
  - Simplest system
  - R1 affects λ_2 wave speed





$$\frac{\partial c_i}{\partial t} = -\frac{j}{\sigma^2}\sum_{k=1}^{N-1}\left[\nu_i \sigma \delta_{ik} - F\,\nu_i c_i z_k(\nu_k - \nu_N)\right]\frac{\partial c_k}{\partial x}$$

$$\partial j / \partial x = 0; \qquad c_N = -\sum_{i=1}^{N-1}\frac{z_i}{z_N}c_i$$

3 species ⇨

Wave relations	Riemann invariants	Wave speeds
$\frac{\partial R_1}{\partial t} = 0$	$R_1 = \sum_{i=1}^{3} z_i c_i / \nu_i$	$\lambda_1 = 0$
$\frac{\partial R_2}{\partial t} - \lambda_2 \frac{\partial R_2}{\partial x} = 0$	$R_2 = c_1 / c_2$	$\lambda_2 = \frac{Fj}{\sigma^2}\nu_1\nu_2\nu_3 R_1$

Bercovici, M. and Santiago, JG, unpublished results

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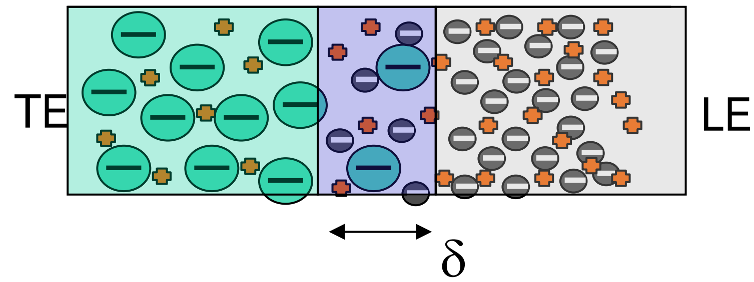
1D Pertubation model



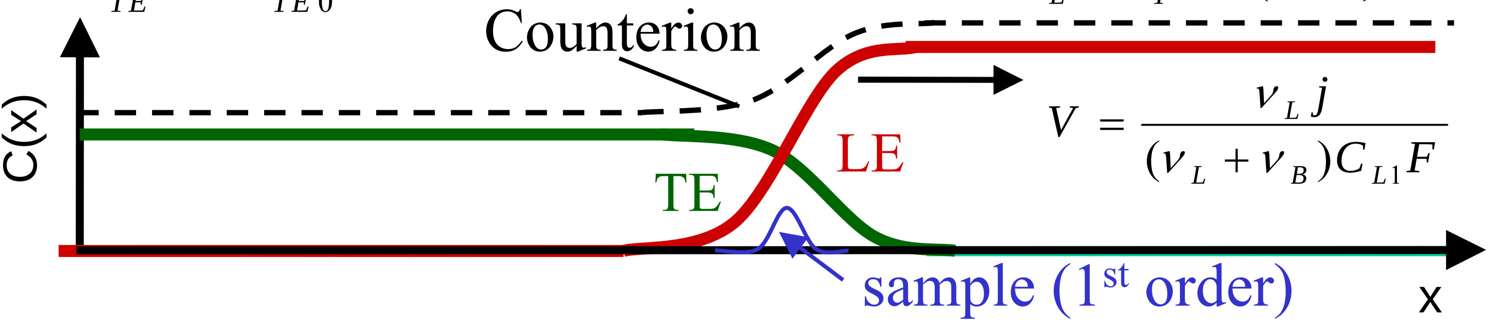
Expand on $\varepsilon = C_X / C_{LE} \sim 10^{-4}$

$$C_i = C_i^0 + \varepsilon C_i^1 + \varepsilon^2 C_i^2 + \ldots \quad E = E^0 + \varepsilon E^1 + \varepsilon^2 E^2 + \ldots$$

0th Order solution:

$$\frac{\partial C_i^0}{\partial t} - V\frac{\partial C_i^0}{\partial X} = \frac{\partial}{\partial X}\left(z_i \nu_i E^0 C_i^0 + D_i \frac{\partial C_i^0}{\partial X} \right) \qquad \left(\frac{\partial C_i^0}{\partial t} \approx 0\right)$$

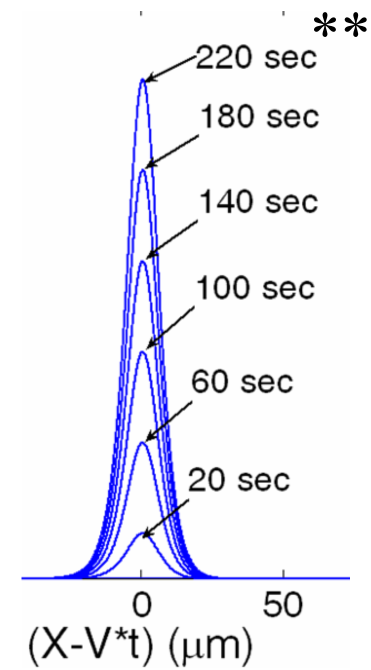
$$\frac{C_{LE}}{C_{TE}} = \frac{C_{LE0}}{C_{TE0}} \exp(x/\delta) \qquad \delta = \frac{\nu_L \nu_T}{\nu_L - \nu_T} \frac{1}{V} \left(\frac{RT}{e}\right)^*$$



$$V = \frac{\nu_L j}{(\nu_L + \nu_B) C_{L1} F}$$

1st Order solution: Sample ion response

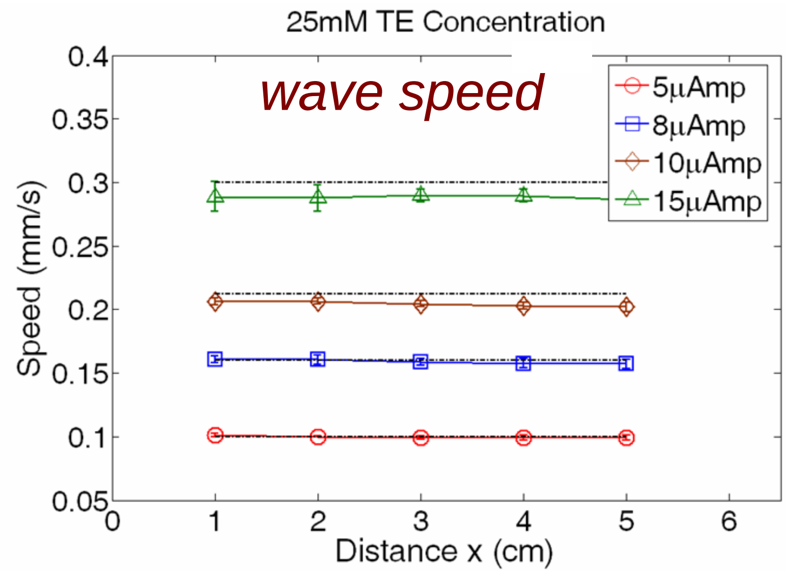
$$\frac{\partial C_X^1}{\partial t} - V\frac{\partial C_X^1}{\partial X} = \frac{\partial}{\partial X}\left(z_X C_X^1 \nu_X E^0 + D_X \frac{\partial C_X^1}{\partial X} \right)^{**}$$

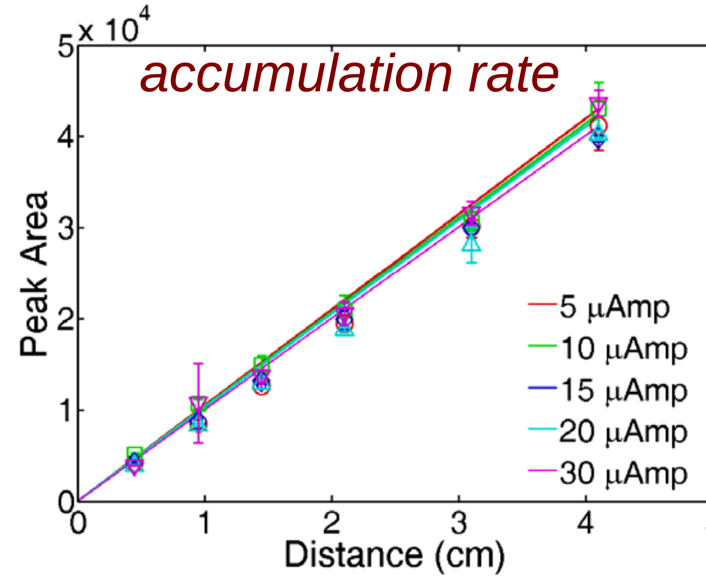


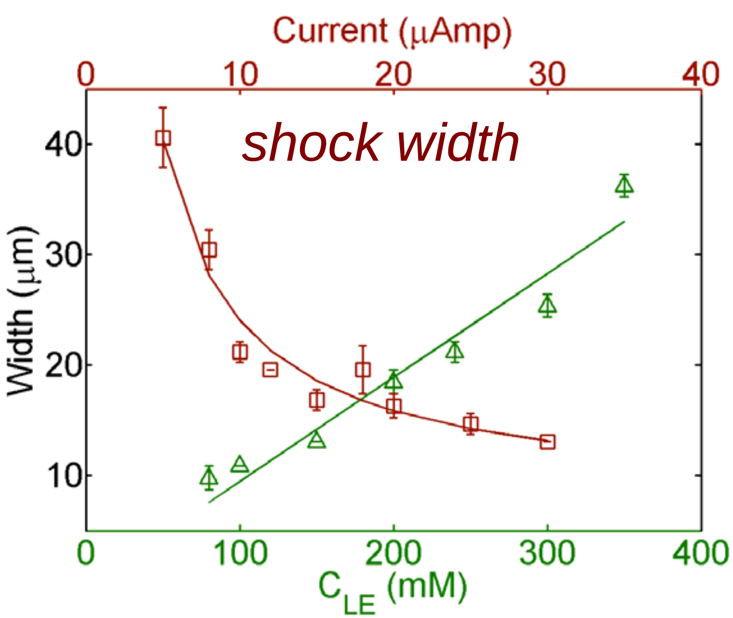
*-Konstantinov (1966)

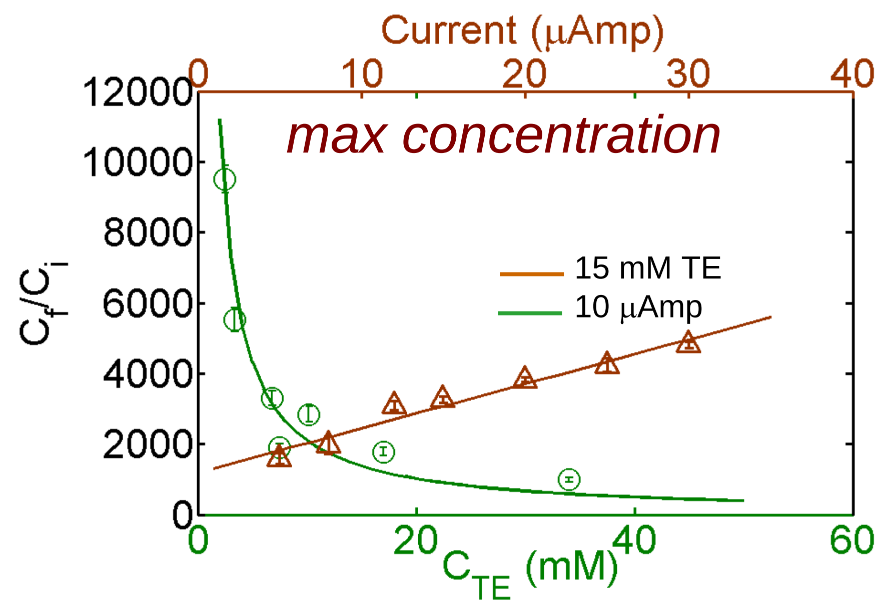
**-Khurana & Santiago, unpublished result (2007)

Pertubation model:* Experimental validation









*-Generalized to include weak electrolyte equilibria

Khurana & Santiago, unpublished result (2007)

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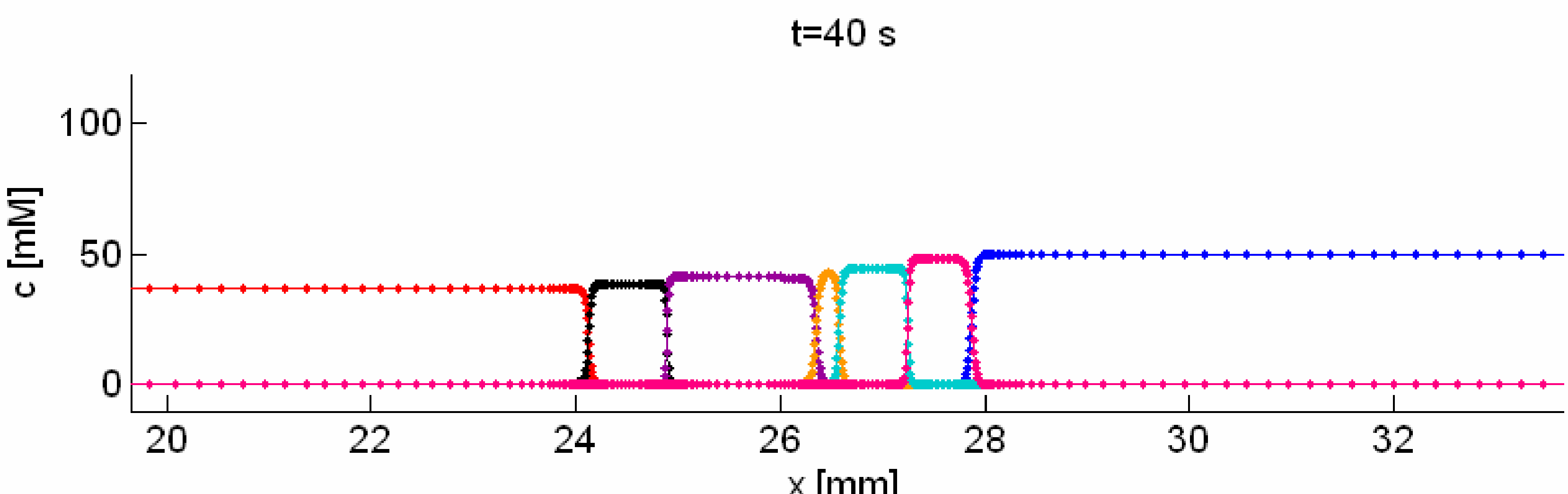
Shock Capture Code for Multi-Species ITP

Collaboration with Sanjiva Lele, Stanford

- Multispecies convection-diffusion solver
  - High resolution (20% resolving efficiency w/ 0.1% accuracy)
  - 4th order in space and time
  - Explicit in time, implicit in space
  - Adaptive grid (~3 min for 10 weak electrolyte ITP focusing/separation)
  - Electric body force
  - Area-averaging, non-uniform EOF, Taylor dispersion (ongoing work)
- Chemical equilibrium for generalized weak electrolytes (300 chemical data base)



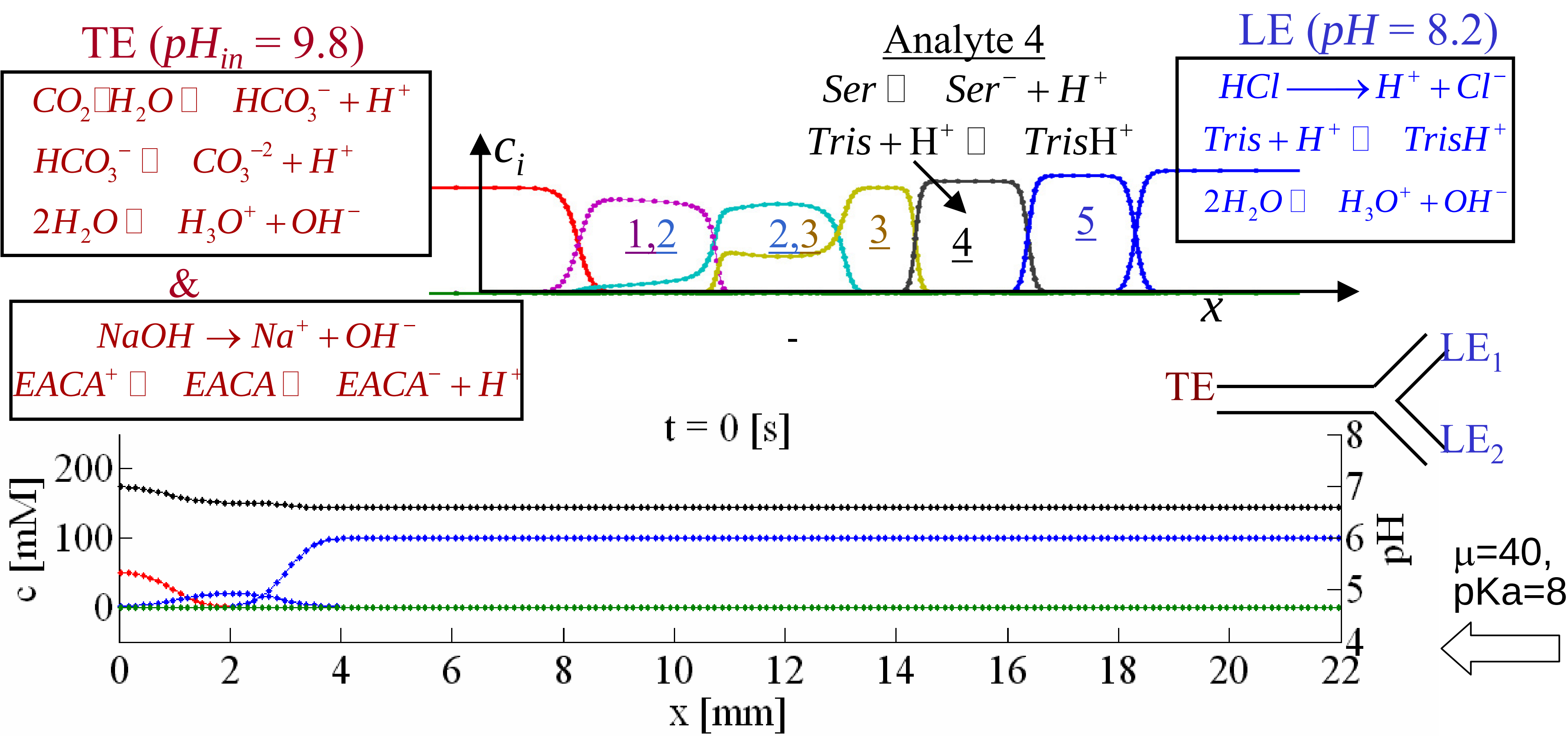
Bercovici, M. and Santiago, JG, unpublished results

ITP of Weak Electrolytes

- Realistic ITP is a coupled convective-diffusion-electromigration-reactions problems:
- Effective (time averaged) mobility

$$\mu_i^{eff} = \sum_{z=n_i}^{p_i} Fz\mu_{i,z}c_{i,z} \Big/ \sum_{z=n_i}^{p_i} c_{i,z}$$

(function of $pH(pKa_1(I), pKa_2(I),.., c_1, c_2, \ldots c_N)$)



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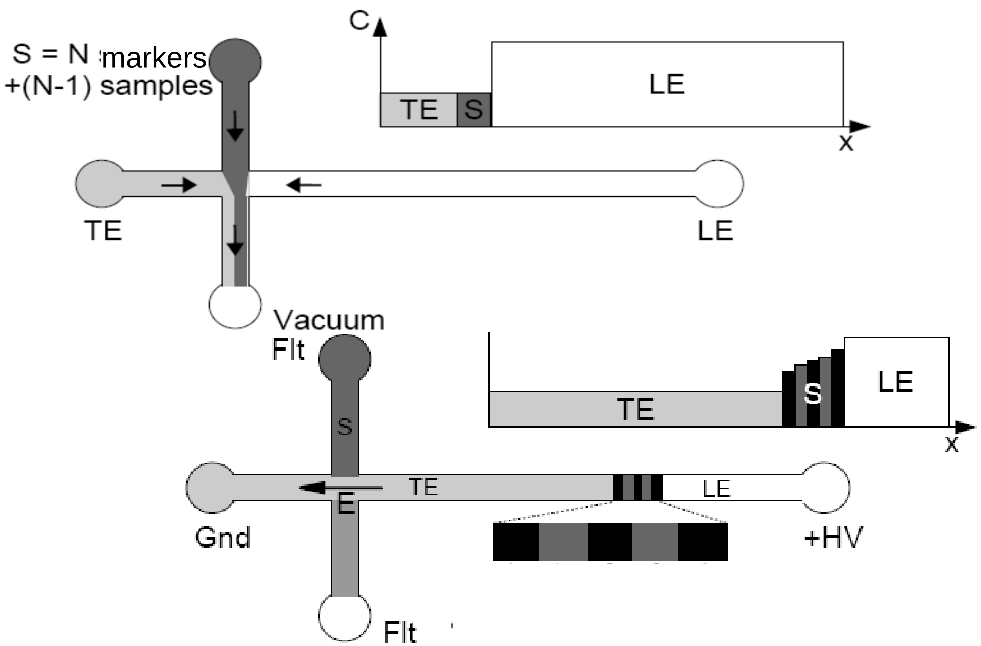
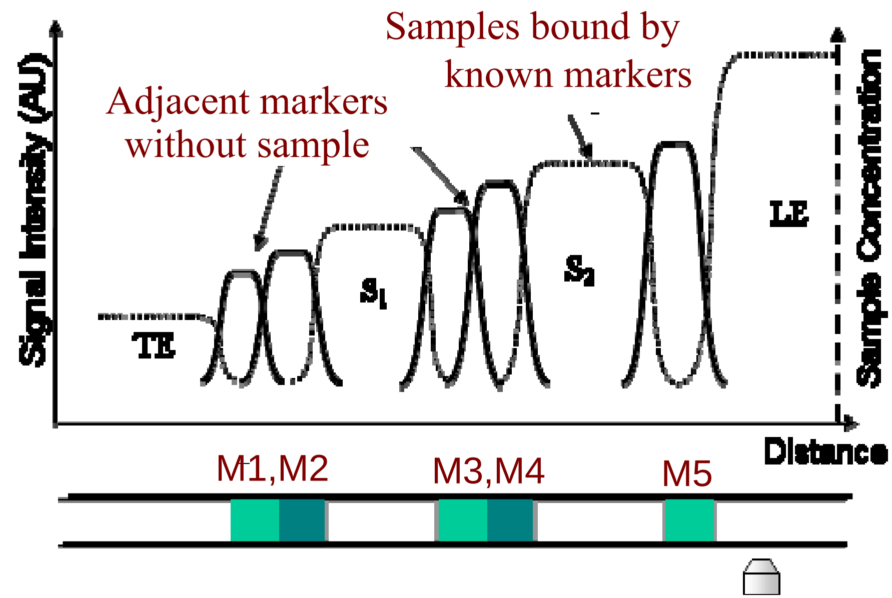
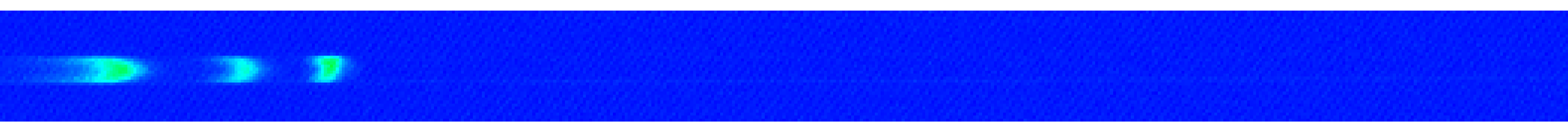
Fluorescent Mobility Markers



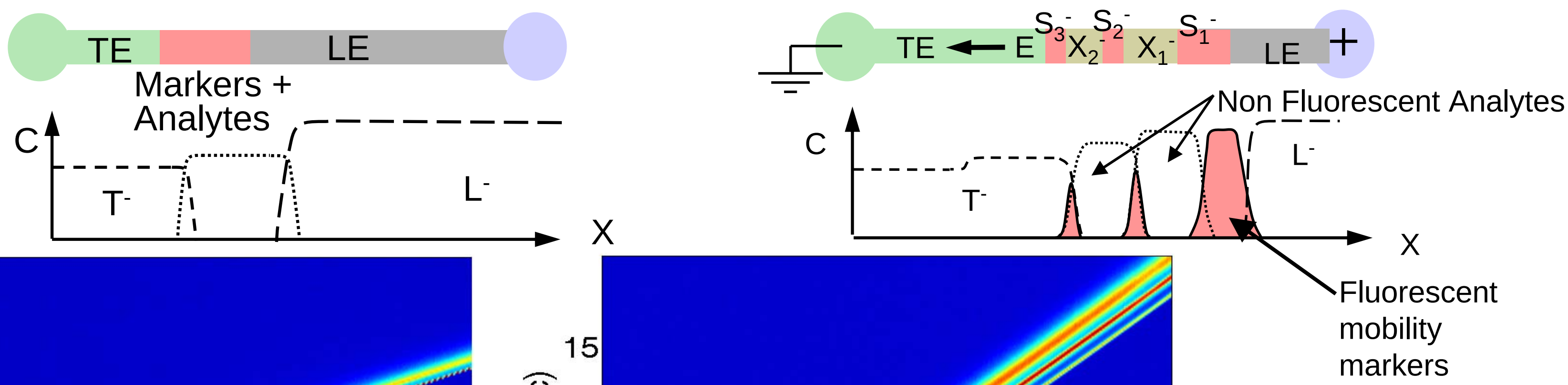


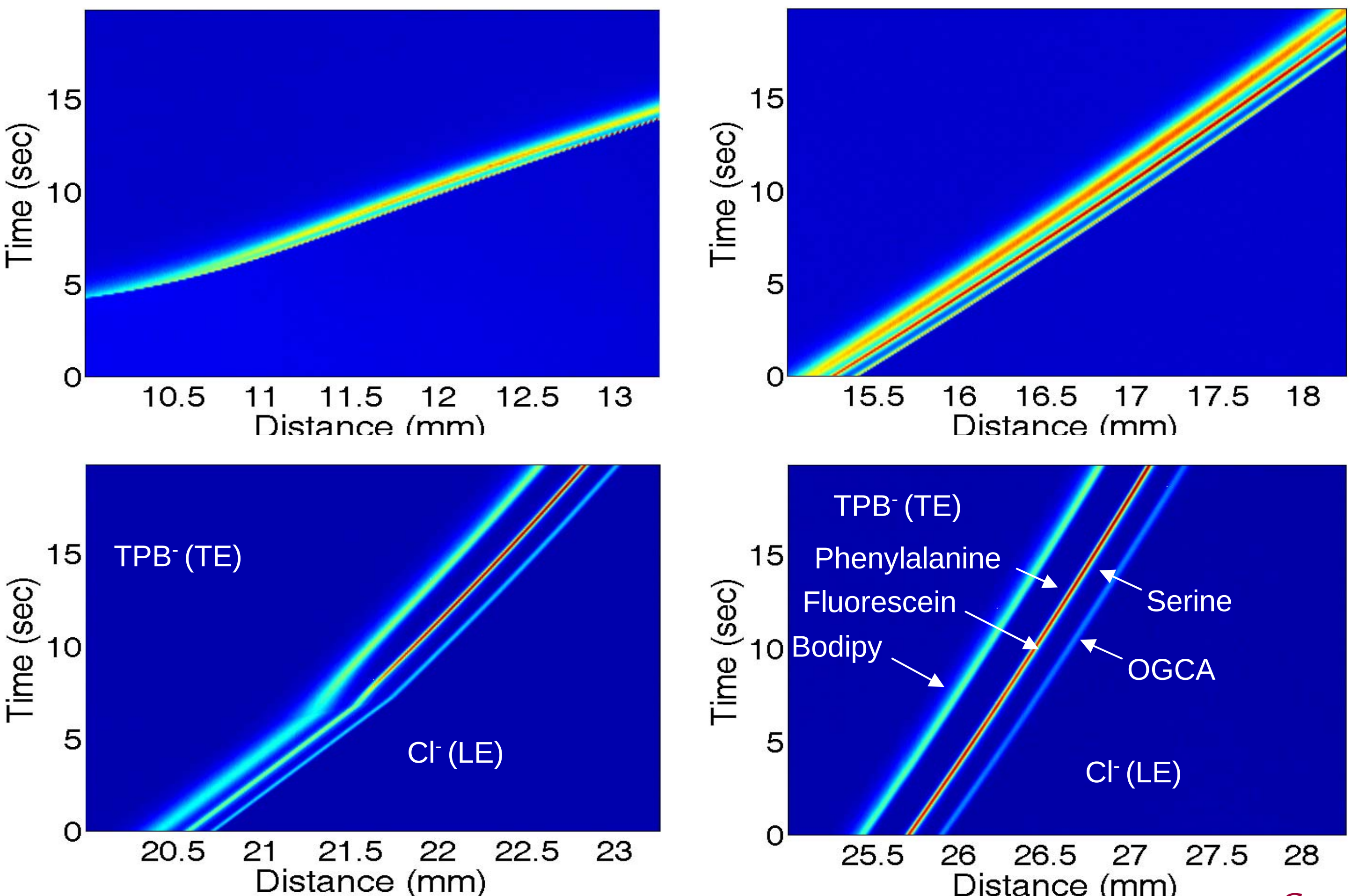
- Series of non-fluorescent analytes sandwhiched between a series of fluorescent mobility markers
- Analyte detectable as “gap” in the mobility markers



Khurana & Santiago, “Pre-Concentration, Separation, and Indirect Detection of Non-Fluorescent Analytes using Fluorescent Mobility Markers,” in press *Analytical Chemistry*, 2007.

Mobility marker experiments





LE: Tris-Chloride
TE: Sodium - TPB (Tetraphenyl-Borate)

X_1: Serine (10 mM)

X_2: Phenylalanine (10 mM)

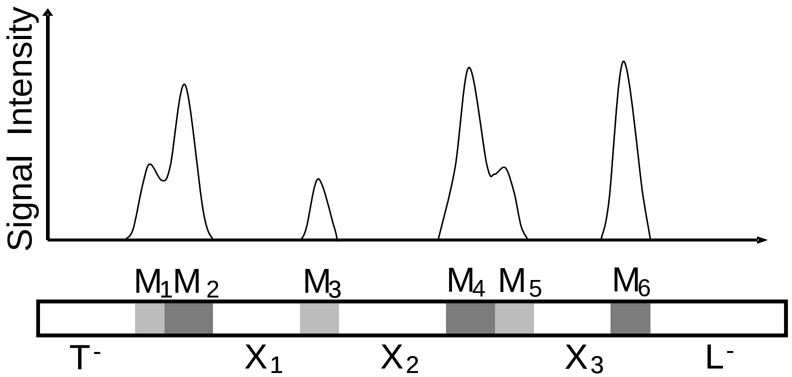
S_1: OGCA (Oregon Green carboxylic acid) (1 um)

S_2: Fluorescein (1 um)

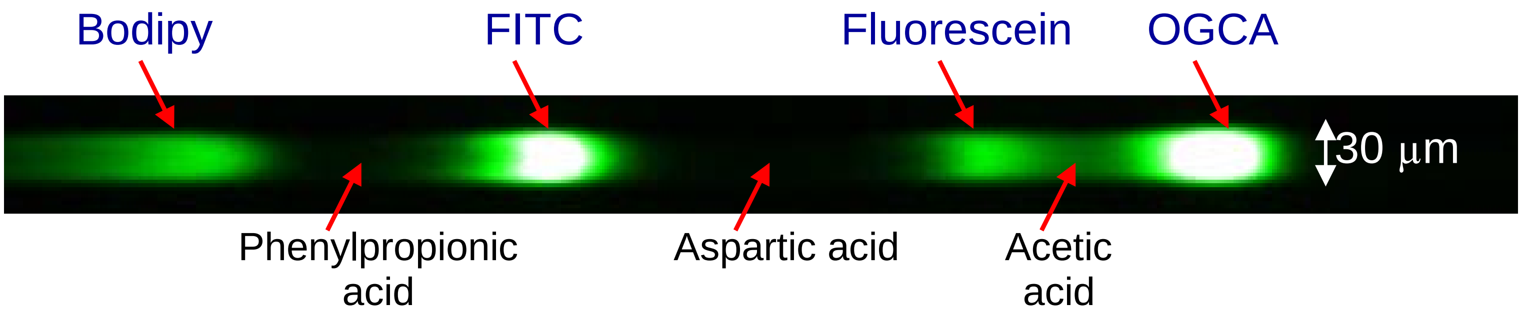
S_3: Bodipy (1 um)

Khurana & Santiago, in press, *Analytical Chemistry*, 2007.

Concentration encoding scheme



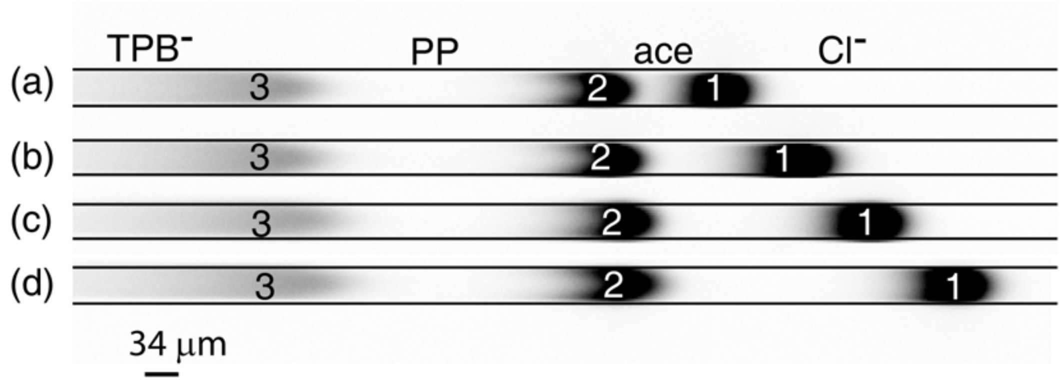
Three analytes/four spacers
High/low/high/low spacer encoding scheme



Experiment Conditions:
Leading electrolyte: 5 mM Tris-HCl, pH 9.1
Trailing electrolyte: 5mM sodium tetraphenylborate.
Analytes: 10 µM acetic acid, 25 µM aspartic acid, 20 µM phenylpropionic acid
Spacers: Oregon Green Carboxylic Acid (OGCA), Fluorescein, Fluorescein Isothiocynate (FITC), Bodipy.

Khurana & Santiago, "Pre-Concentration, Separation, and Indirect Detection of Non-Fluorescent Analytes using Fluorescent Mobility Markers," in press, *Analytical Chemistry*, 2007.

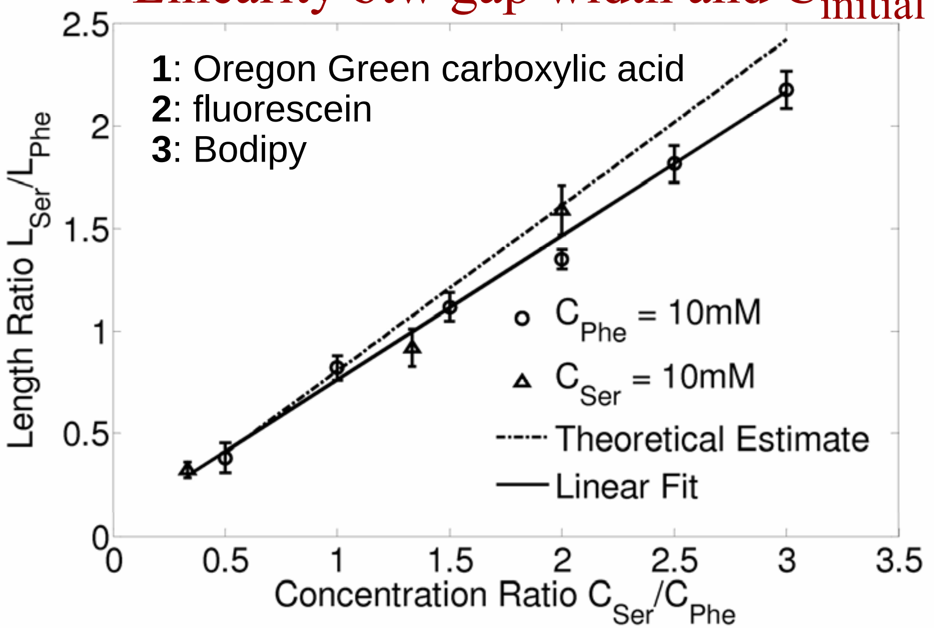
Linear detector of concentration



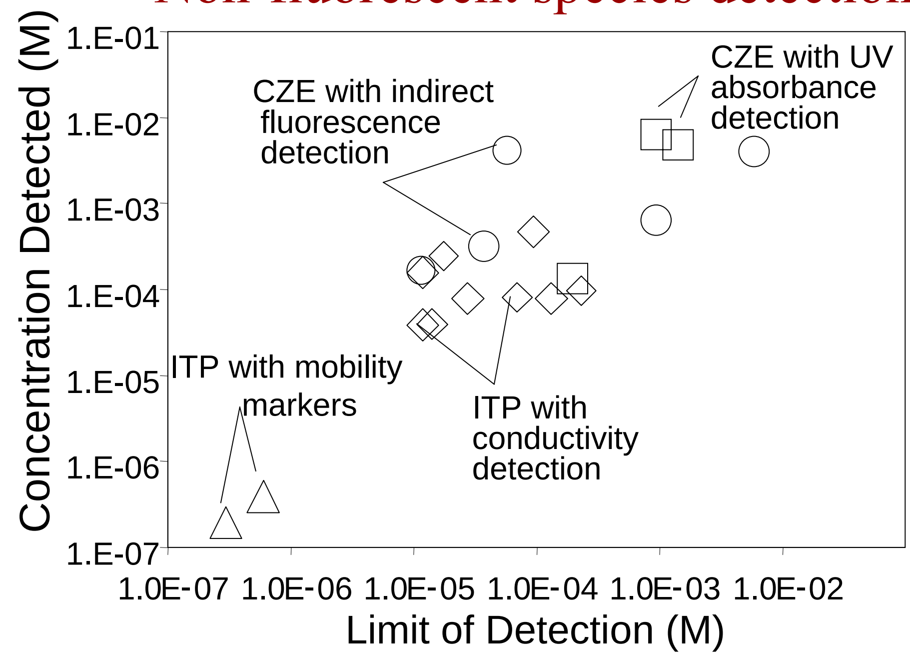
- C_{ace} = 12 to 48 μM
- C_{PP} = 40 μM

Demonstrated detection of simple acids, amino acids, proteins, and DNA
Current limit: 100 nM

Linearity btw gap width and $C_{initial}$

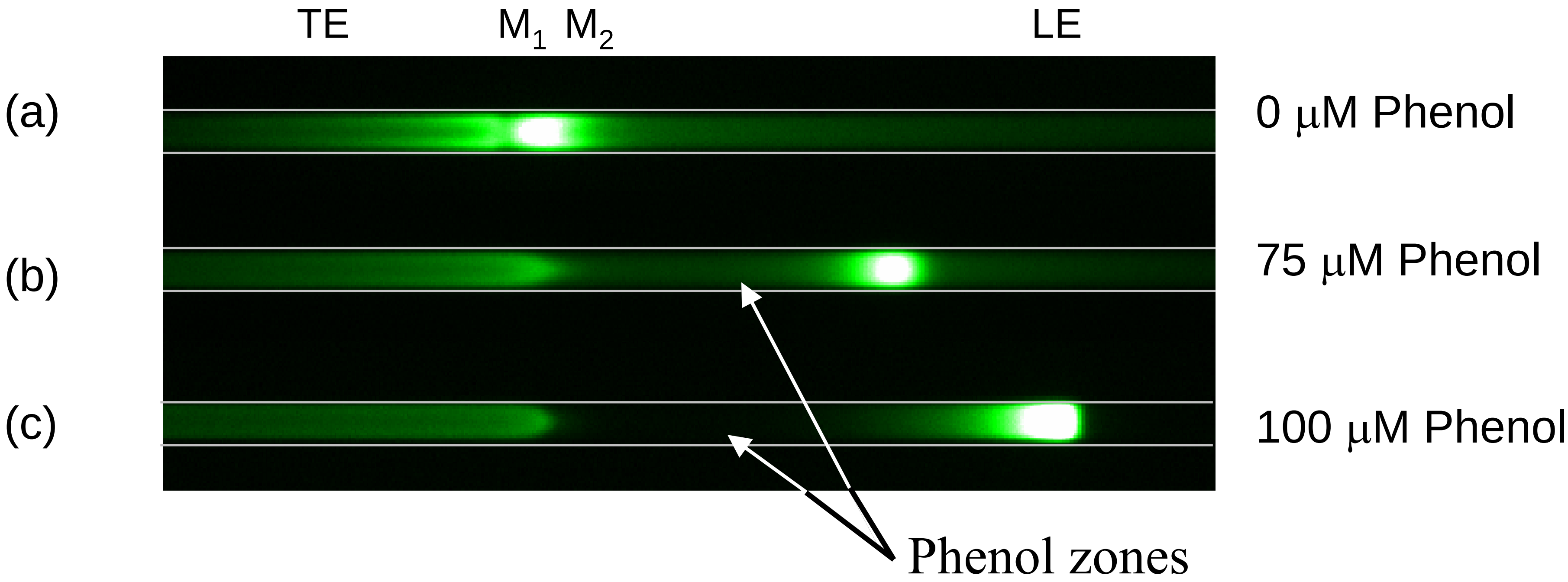


Non-fluorescent species detection



Khurana & Santiago, "Pre-Concentration, Separation, and Indirect Detection of Non-Fluorescent Analytes using Fluorescent Mobility Markers," in press, *Analytical Chemistry*, 2007.

Detection of Phenol Using Fluorescent Mobility Markers



LE: Tris-Acetate (25 mM, pH 9.8)

TE: Tris-EACA (epsilon amino caproic acid, 25 mM, pH 9.2)

Mobility Markers: M_1 50 nM Fluorescein,

M_2 50 nM Dextran Alexa Fluor (MW 10,000)

Outline

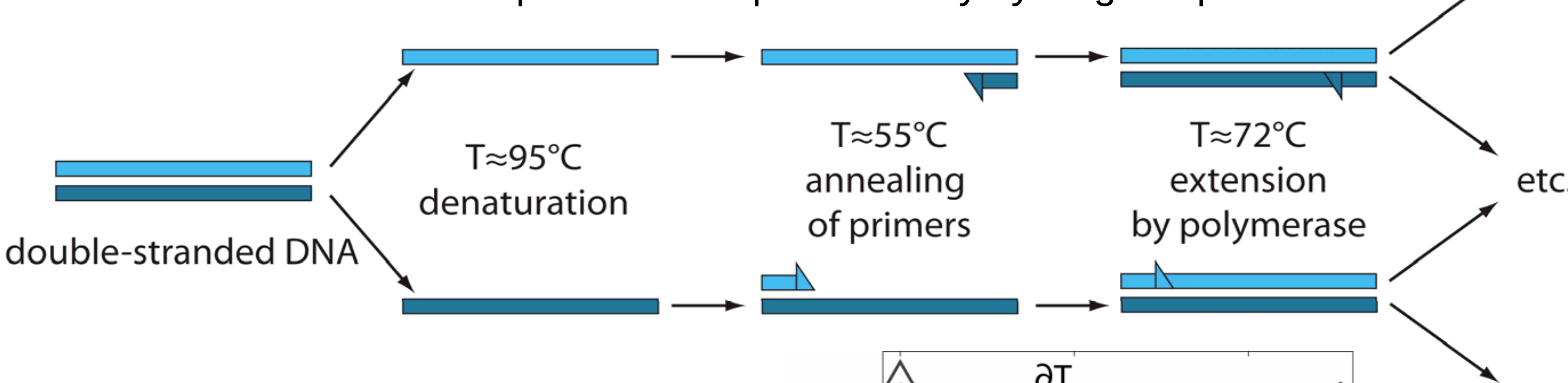
- Background: Microfluidics and isotachophoresis (ITP)
- ITP process and visualizations
- ITP models
  - Perturbation model
  - Shock capturing code for multispecies with reactions
- ITP applications
  - Fluorescence detection of non-fluorescent analytes
  - Isothermal PCR for DNA amplification
- Summary and near future work

The polymerase chain reaction (PCR)

In-vitro DNA amplification technique

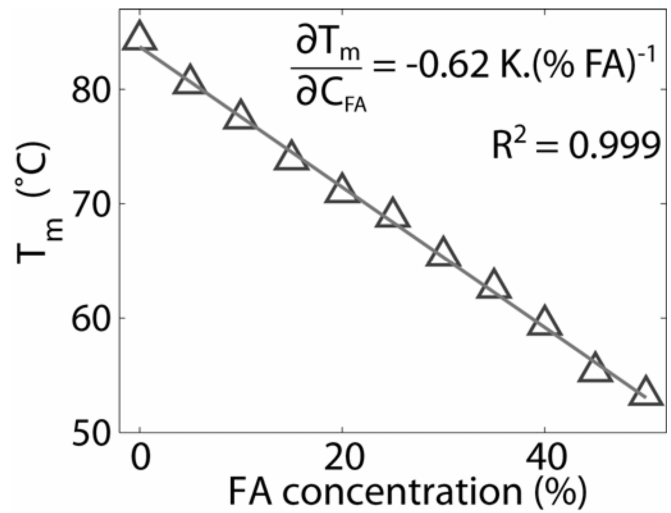
- ~400,000+ j. pubs with "PCR" (one review[1] cited ~ 15,000 times)
- An essential tool in molecular biology and medical research

Traditional PCR: exponential amplification by cycling temperature



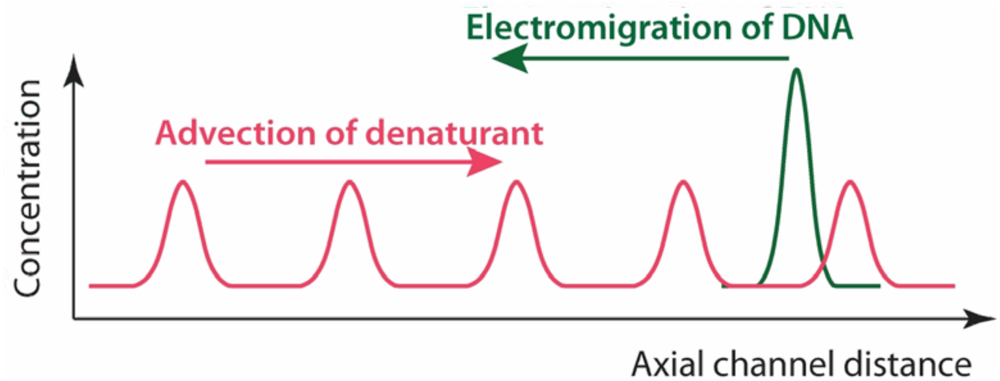
DNA melting temperature depends strongly on solvent:

In 50% formamide (v/v), DNA can melt at 55°C

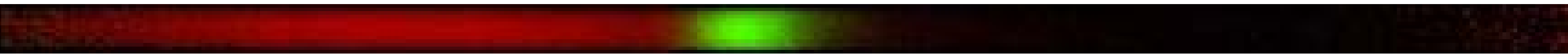


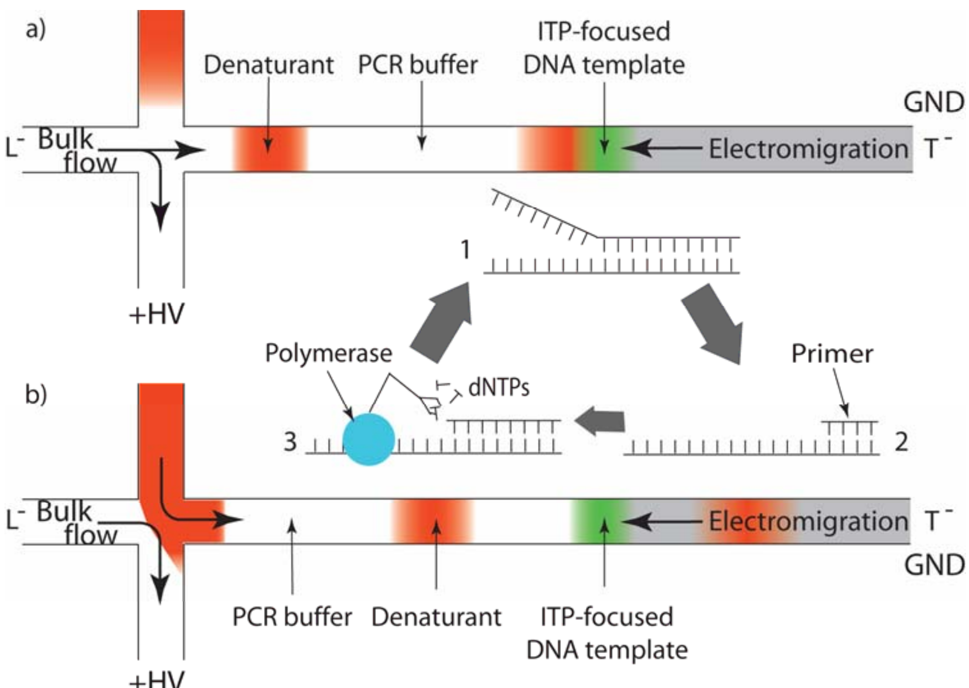
[1]Saiki R.K. *et al.*: *Primer-directed enzymatic amplification of DNA with a thermostable DNA-polymerase.* Science, 1988.

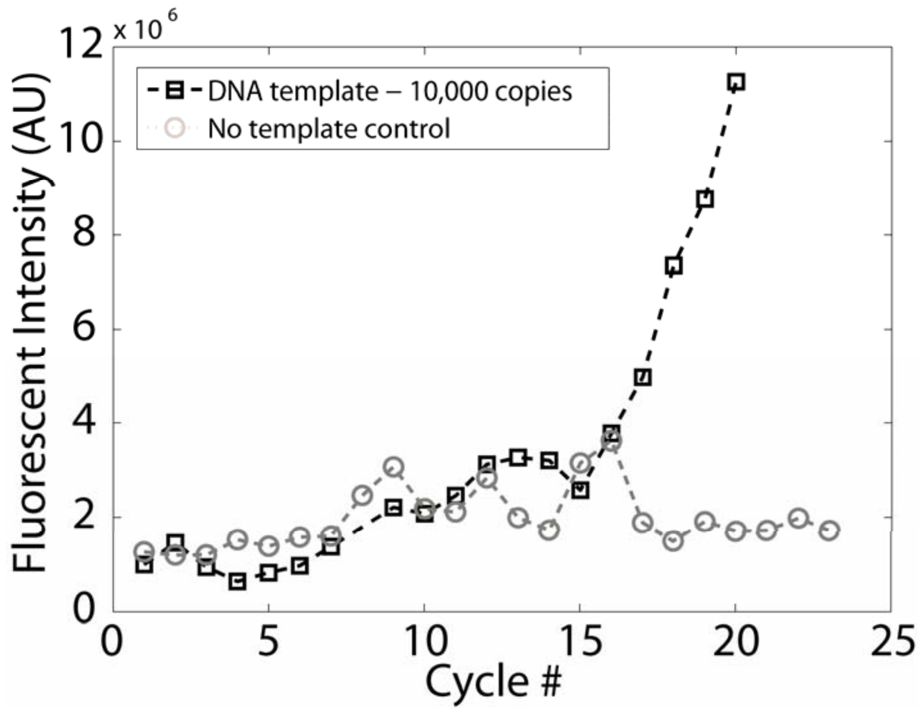
Isothermal PCR (iPCR)



- DNA electromigration via ITP:
  DNA is focused (limited dispersion)
- Counterflow:
  DNA stationary, advects denaturant







Collaboration with Ebara Corporation
Persat, Morita, Santiago, uTAS 2007 Conference

Summary

- Models capture essential physics, provide design tools for optimization
- Enables indirect detection of unlabeled analytes
  - ~50 nM sensitivity
  - Applying to detection of toxins and chemical weapons
- Enables isothermal PCR
  - Currently optimizing PCR conditions
  - Developing quantitative (real time) PCR
- Near future work
  - Novel indirect detection methods
  - Protein ITP

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- On-chip ITP
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  - Raymond Sierra
- Nanochannels
  - Tom Zangle
- Miniature fuel cells
  - Shawn Litster
  - Cullen Buie
  - Dr. Tibor Fabian
  - Dan Strickland

Recent Alumni (2002 to 2007):

- Prof. Chuan-Hua Chen, Duke U.
- Prof. Hao Lin, Rutgers U.
- Prof. Jonathan Posner, Arizona State U.
- Prof. Sumita Pennathur, UC Santa Barbara
- Prof. Amy Herr, UC Berkeley
- Prof. Guiren Wang, USC
- Dr. Shankar Devasenathipathy, Intel Corp.
- Dr. Michael Oddy, Barclays Corp.
- Dr. Klint Rose, Lawrence Livermore Natl. Labs
- Dr. David Huber, Sandia National Labs
- Dr. Byoungsok Jung, Sandia National Labs
- Dr. Rajiv Bharadwaj, Caliper Technologies
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